AD

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 :		(11) International Publication Number:	WO 94/02107
A61K	A2	(43) International Publication Date:	3 February 1994 (03.02.94)

(21) International Application Number: PCT/US93/06933

(22) International Filing Date: 23 July 1993 (23.07.93)

(30) Priority data:
07/919,309   24 July 1992 (24.07.92)   US

(71)(72) Applicant and Inventor: KENNEDY, Thomas, P. [US/US]; McGuire Clinic, 7702 Parham Road, Richmond, VA 23294 (US).

(74) Agent: PERRYMAN, David, G; Needle & Rosenberg, 127 Peachtree Street, N.E., Suite 1200, Atlanta, GA 30303 (US).

(81) Designated States: AU, BR, CA, JP, KR, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: METHOD AND MEDICAMENT FOR INHIBITING NEUTROPHIL ELASTASE AND CATHEPSIN G

(57) Abstract

A method and medicament for the inhibition of neutrophil elastase and cathepsin G in mammals comprising administering a treatment effective amount of 2-O-desulfated heparin to a mammal in need thereof. The medicament preferably is administered by aerosolization or by intravenous (IV) injection. In specific embodiments of the invention, the ratio of 2-O-desulfated heparin to elastase is greater than about 0.2 and less than about 2.0, or the ratio of 2-O-desulfated heparin to cathepsin G is greater than about 0.4 and less than about 2.0. Preferably, the 2-O-desulfated heparin medicament includes a physiologically acceptable carrier which may be selected from the group consisting of physiologically buffered saline, normal saline, and distilled water.

METHOD AND MEDICAMENT FOR INHIBITING NEUTROPHIL ELASTASE AND CATHEPSIN G

5   The present invention relates to medical treatment of mammals and more specifically to methods and medicaments for the control of neutrophil elastase and cathepsin G in mammals.

Activated neutrophils play an important role in
10   a number of human and other mammalian diseases by releasing a number of oxidant chemicals and enzymes after migration into an affected organ. While oxidants, such as superoxide anion, hydrogen peroxide and hypochlorous acid are injurious by themselves, the major destructive elements
15   produced by activated neutrophils are cationic proteases, the bulk of which consist of elastase and cathepsin G. When neutrophils release these proteases, tissue destruction occurs unless the proteases are neutralized by sufficient extracellular anti-proteinases such as α-1-anti-
20   proteinase.

Individuals with an inherited deficiency of α-1-anti-proteinase suffer unimpeded proteolytic lung destruction over a lifetime, resulting ultimately in the development of pulmonary emphysema. Cigarette smoking
25   causes the influx of activated leukocytes into the lung, with subsequent degranulation and release of proteases. Cigarette derived oxidants also inactivate α-1-anti-proteinase by oxidizing an important methionine near the active site. Elastase delivered to the alveolar lung unit
30   as a result of the influx due to cigarette smoking, concurrent with oxidative inactivation of α-1-anti-proteinase activity, produces an imbalance of protease/anti-proteinase activity that is thought to be a major cause of human emphysema from cigarette smoking.

35   When the imbalance occurs within the airway, chronic airway inflammation is the result, and neutrophil derived elastase and cathepsin G are thought important in the pathogenesis of chronic bronchitis. If the imbalance occurs within the pulmonary vasculature, the resulting
40   microvascular injury causes lung edema formation. In this fashion the influx of activated leukocytes and release of

elastase and other neutrophil proteases are major causes of
lung injury in the Adult Respiratory Distress Syndrome.
Neutrophil derived elastase is also an important cause of
proteolytic lung destruction in cystic fibrosis, a disease
5   characterized by intense mucopurulent bronchitis and some
of the highest levels of elastase activity measured in any
human disease.

Also, diseases such as myocardial infarction and
stroke, caused by sudden loss of organ blood flow, followed
10   by blood flow restoration (ischemia-reperfusion injury) are
characterized by magnification of tissue destruction during
the reperfusion phase when activated leukocytes rapidly
invade the already injured tissue. Neutrophil elastase
delivered to ischemic reperfused organs has been
15   demonstrated to play a pivotal role in the pathogenesis of
reperfusion injury of the myocardium, bowel and other
tissues. The role of cathepsin G in the processes above is
not as well studied, but may be equally important, since
there is twice as much cathepsin G present in the
20   neutrophil as elastase.

Because elastase and cathepsin G are mediators of
a variety of important human diseases, developing effective
inhibitors of these enzymes is an active goal in
experimental pharmacology. However, to date, no completely
25   effective and safe inhibitor of both elastase and cathepsin
G exists. A small organic inhibitor of elastase has been
developed (C.P. Sommerhoff, et al., European Journal of
Pharmacology (1991) 193:153-158), but it failed to
demonstrate activity against cathepsin G. Two
30   biomolecules, α-1-anti-proteinase inhibitor and bronchial
secretory inhibitor, are sensitive to inactivation by
neutrophil oxidants and are not likely to be effective in
biologic environments where neutrophil oxidants and
proteases are present simultaneously (D. C. Flenley,
35   Quarterly Journal of Medicine (1986) 61:901-909; C.
Vogelmeier, et al., Journal of Clinical Investigation
(1991) 87:482-488). An inhibitor is needed which is
simultaneously effective against both elastase and

cathepsin G but is impervious to either proteolytic or oxidative inactivation. The sulfated polysaccharides have each of those desirable qualities.

It has been previously reported that heparin and other sulfated polysaccharides are potent non-competitive inhibitors of elastase and cathepsin G from human polymorphonuclear leukocytes (N. V. Rao, et al., A. M. Rev. Respir. Dis. (1990) 142:407-412; A. Baici, et al., Biochem. Pharmacol. (1980) 29:1723-1727; A. Baichi, et al., Biochem. Pharmacol. (1981) 30:703-708; K. Marossy, Biochim. Biophys. Acta. (1981) 659:351-361; A. Bacici, et al., Chem-Biol. Interactions (1984) 51:1-11; A. Lutini, et al., Biochem. Int. (1985) 10:221-232; F. Redini, et al., Biochem. J. (1988) 252:515-519; and F. Redini, et al., Biochem. Pharmacol. (1988) 37:4257-4261.) It is believed that the basis for inhibition is by formation of electrostatic bonds between the negatively charged sulfate groups of the polysaccharide and the positively charged guanidinium groups of the arginine residues located at the surface of those highly basic enzymes such as elastase or cathepsin G. The interaction does not influence the active center of the enzyme but causes an indirect loss of elastolytic activity.

Of all the sulfated polysaccharides, heparin has the longest and safest history of use in man. From a toxocologic consideration, heparin is the most desirable inhibitor of elastase and cathepsin G but for the fact that it is an anti-coagulant even when delivered selectively to the lung by aerosol. It is believed that heparin acts as an anti-coagulant because of a repeated sequence of saccharides which binds specifically to the plasma protein anti-thrombin III, dramatically accelerating the rate at which anti-thrombin inhibits the procoagulant effect of thrombin on the cascade of blood coagulation. Only a portion of commercial heparin binds to anti-thrombin III, and passage of heparin over an affinity column of anti-thrombin III-sepharose removes the anti-coagulant fraction leaving an incompletely sulfated fraction devoid of anti-

4

coagulant activity. However, utilizing this process to rid heparin of its anti-coagulant activity is too inefficient to be undertaken on a commercially practical scale.

In the prior art, it has been noted that the activity of a polysaccharide as an inhibitor of human polymorphonulear leucocyte elastase (HLE) and cathepsin G is directly dependent upon the presence of intact sulfate groups. Dextran sulfate is a potent inhibitor of elastase, but non-sulfated dextran is not. Further-more, the available literature suggests that even partial desulfation of polysaccharides eliminates inhibitory activity toward HLE and cathepsin G while chemical oversulfation enhances inhibitory activity. The importance of sulfate groups was studied using fragments of heparin obtained by chemical depolymerization with HNO_2 followed by gel filtration (F. Redini, et al., Biochem. J., (1988) 252:515-519). Unmodified heparin fragments obtained by this latter process were potent inhibitors of elastase but retained their strong anticoagulant power. Increasing the degree of sulfation by chemical O-sulfation of the fragments markedly increased their potency as elastase inhibitors but did not materially alter the anticoagulant activity of the fragments. On the other hand, N-desulfation followed by N-acetylation (to cover the remaining positive charge and reduce the anticoagulant activity of the fragments) completely eliminated inhibitory activity toward human leukocyte elastase and cathepsin G. Chemical over-O-sulfation of the N-desulfated fragments not only restored inhibitory activity but gave the fragments higher inhibitory potential compared to their original counterparts with a similar degree of sulfation but containing N-sulfate groups. It has been suggested that not only was the degree of sulfation important to inhibitory activity, but that the presence of O-sulfates were more important than the presence of N-sulfates. However, none of these highly effective, modified heparins were suitable for use in mammals due to their potent continuing anticoagulant activity.

Several chemical methods exist for inactivating heparin as an anti-coagulant. Most are based on techniques of chemical desulfation, since it is well established that degree of sulfation is an important determinant of anticoagulant activity.

N-desulfation by treatment of the pyridinium heparin salt with dimethylsulfoxide (DMSO) in five percent methanol for 1.5 hours at 50°C and total desulfation by similar treatment in DMSO in 10% methanol for 18 hours at 100°C are commonly used chemical modifications to remove anti-coagulant activity from heparin. Another method to remove anti-coagulant activity from heparin is acid hydrolysis at 55-60°C for 72 hours to produce partial N-desulfation. However, removal of all sulfates or even a partial desulfation by removal of N-sulfates inactivates heparin and other sulfated polysaccharides as inhibitors of human elastase and cathepsin G. Thus, the prior art teaches that currently utilized desulfation methods which remove anti-coagulant activity of heparin also destroy its ability to inhibit cationic leukocyte proteases such as elastase and cathepsin G.

Thus, the prior art demonstrates that over-sulfation leads to increased activity against elastase and cathepsin G with continuing anticoagulant activity while desulfation leads to decreased anticoagulant activity with greatly diminished activity against elastase and cathepsin G. In contrast to what would be predicted by the prior art, the present inventors have discovered that selective 2-O-desulfation of α-L-iduronic acid-2-sulfate eliminates the anticoagulant activity of heparin without destroying the activity of the modified heparin as an inhibitor of elastase and cathepsin G.

It is an object of the present invention to provide a method for inhibiting elastase and cathepsin G in mammals.

It is another object of the present invention to provide such inhibition in a therapeutic agent which substantially does not induce anti-coagulant activity.

6

It is a further object of the present invention to provide a method for inhibiting elastase and cathepsin G by aerosol treatment or intravenous (IV) administration of the therapeutic agent.

It is an advantage of the present invention that the method provides a therapeutic agent which substantially inhibits elastase and cathepsin G without inducing anti-coagulant activity.

It is a further advantage of the present invention that the therapeutic agent is produced from a toxicologically characte-rized compound.

Consideration of the specification, including the several figures and examples to follow will enable one skilled in the art to determine additional objects and advantages of the invention.

The present invention provides a medicament for the inhibition of neutrophil elastase and cathepsin G in mammals comprising a treatment effective amount of desulfated heparin prepared by the removal of the 2-O-sulfate of α-L-iduronic acid-2-sulfate saccharide units of heparin. In preferred embodiments of the invention, the medicament is administered by aerosolization or by intravenous (IV) injection. In other embodiments of the invention, the effective ratio of 2-O-desulfated heparin to elastase is chosen to be greater than about 0.2 and less than about 2.0, or effective the ratio of 2-O-desulfated heparin to cathepsin G is chosen to be greater than about 0.4 and less than about 2.0. Preferably, the medicament includes a physiologically acceptable carrier which may be selected from the group consisting of physiologically buffered saline, normal saline, and distilled water.

The present invention also provides a method for inhibiting neutrophil elastase and cathepsin G in mammals comprising administering a treatment effective amount of 2-O-desulfated heparin to a mammal. In preferred embodiments of the method of the invention, the treatment effective amount is administered by aerosolization or by intravenous

(IV) injection. In other embodiments of the invention, the
effective ratio of 2-0-desulfated heparin to elastase is
greater than about 0.2 and less than about 2.0, or the
effective ratio of 2-0-desulfated heparin to cathepsin G is
5   greater than about 0.4 and less than about 2.0.
Preferably,the medicament includes a physiologically
acceptable carrier which may be selected from the group
consisting of physiologically buffered saline, normal
saline, and distilled water.

10   Reference to the following detailed description
may help to better explain the invention in conjunction
with the drawings in which:

          FIGURE 1 shows a chemical formula of 2-0-
desulfated α-L-iduronic acid in the pentasaccharide binding
15   sequence of heparin;

          FIGURE 2 shows a proposed reaction scheme for
desulfating the 2-0-position of α-L-iduronic acid in the
pentasaccharide binding sequence of heparin;

          FIGURE 3 shows a graph of the number of
20   polymorpho-nuclear leukocyte (PMN) cells in the
bronchoalveolar lavage fluid 24 hours after the
administration of saline (control) human leukocyte elastase
(HLE), HLE plus heparin, and HLE plus desulfated heparin;

          FIGURE 4 shows a graph of the hemoglobin content
25   measured in the bronchoalveolar lavage fluid 24 hours after
the administration of saline (control), HLE, HLE plus
heparin, and HLE plus desulfated heparin; and

          FIGURE 5 shows a graph of the concentration of
protein in the bronchoalveolar lavage fluid 24 hours after
30   administration of saline (control), HLE, HLE plus heparin,
and HLE plus desulfated heparin.

          The removal of the 2-0-sulfate from α-L-iduronic
acid in the pentasaccharide binding sequence of heparin,
shown in FIGURE 1, occurs due to the chemistry of sulfated
35   carbohydrates in alkaline conditions which was first
elucidated by E. G. V. Percival, Quarterly Rev., (1949)
3:369-384, and reviewed in J. P. R. Turvey, Adv.
Carbohydrate Chem., (1965), 20:183-218. A sulfate group on

8

a secondary hydroxyl group is susceptible to alkaline
hydrolysis if there is an adjacent, _trans_, free hydroxyl
group available for forming an epoxide intermediate as the
sulfate is eliminated. This is shown in FIGURE 2. For
5 heparin, this reaction has been recently reported to occur
with alkaline hydrolysis during lyophilization. (R. Rej,
et al., _Thromb. Haemostas._ (1989) 61:540; and M. Jaseja, _et
al._, _Can. J. Chem._ (1989) 67:1449-1456.) Modification of
these procedures by the present invention produced a
10 heparin which is effective against elastase and cathepsin
G but without the anti-coagulating properties of untreated
heparin.

In order to facilitate a further understanding of
the invention, the following examples primarily illustrate
15 certain more specific details thereof.

<u>EXAMPLE I</u>

<u>Partial N-Desulfation of Heparin</u>

Porcine intestinal mucosal heparin (Scientific
Protein Laboratories, Waunakee, WI) was converted to
20 heparinic acid by passing a 4% aqueous solution over a
cation exchange resin column, Dowex 50W x 8X (H⁺). The
solution of heparinic acid was then kept at 55°C for 72
hours in a standard chemical reflux apparatus to remove
approximately 70% of N-sulfates, as reported by L. Levy _et
25 al._, _Proc. Soc. Exp. Biol. Med._ (1962) 109:901-905 and E.
Sache _et al._, _Thromb. Res._ (1989) 55:247-258. The N-
desulfated heparin was recovered by passing the solution
over IR-400 resin (OH⁻) to remove excess SO₄⁻ followed by
adjustment of pH to 7.0 and lyophilization.

30   <u>EXAMPLE II</u>

<u>Interaction of Partially N-Desulfated Heparin with HLE</u>

The inhibition of HLE by partially N-desulfated
heparin of Example I was measured by incubating a constant
amount of HLE (100 pmol) with increasing amounts of
35 partially N-desulfated heparin (10-50 pmol, I:E ratio 0.1-
0.5) for 30 minutes at 25°C in 500 microliters of Hepes
buffer (0.125 M, 0.125% Triton-X-100, pH 7.5) diluted to
the final volume of 900 microliters. The remaining enzyme

activity was measured by adding 100 microliters of 3mM N-Suc-Ala-Ala-Val-NA (Sigma Chemicals, St. Louis, MO, made in dimethylsulfoxide [DMSO]) and reading the absorbance of the proteolytically released chromogen 4-Nitroaniline at 405 nm. The percentage of inhibition was calculated based on enzyme activity without inhibitor.

The results of the interaction of partially N-desulfated heparin and heparin with HLE are shown in Table I.

TABLE I
PERCENT INHIBITION OF HLE

Substrate Inhibitor*	H	NDH
Ratio I:E		
0.1:1	53	29
0.2:1	79	37
0.3:1	88	37
0.4:1	90	49
0.5:1	91	50

*H = Heparin; NDH = Heparin partially N-desulfated according to Example I

As shown in Table I, heparin significantly inhibited human leukocyte elastase (HLE) at I:E ratios of greater than 0.2. In contrast, heparin partially N-desulfated according to Example I had greatly reduced ability to inhibit HLE, even at I:E ratios of 0.5. Thus, the activity of heparin as an inhibitor of HLE was substantially diminished by even partial desulfation of the polysaccharide by this treatment commonly used to eliminate the anticoagulant effect of heparin. These results were consistent with previously discussed prior art suggesting that desulfation destroys the activity of heparin as an inhibitor of HLE and cathepsin G.

EXAMPLE III
2-O-Desulfation of Heparin:

An aqueous solution of 0.4% porcine intestinal mucosal heparin (Scientific Protein Laboratories, Waunakee,

WI) (4 mg/ml) was adjusted to pH 13.0 with 0.1 N NaOH, frozen, then lyophilized in 40 ml aliquots. After the product was redissolved in water and passed over an Amberlite IR-120 plus (H+) cation exchange resin to remove excess sodium hydroxide, final pH was adjusted to 7.0, and the solution was passed through a 0.2 micron Millipore filter by vacuum filtration to ensure bacterial sterility prior to final re-lyophilization to dryness.

For commercial preparation of larger amounts of the 2-0-desulfated heparin, aqueous solutions (up to 5% weight/volume) of porcine intestinal mucosal heparin (Scientific Protein Labora-tories, Waunakee, WI), sodium salt, are adjusted to pH 13.0 by addition of NaOH, followed by lyophilization. To remove excess NaOH, the dry product is redissolved in aqueous solution, and heparin is precipitated by the addition of three volumes of cold (4°C) ethanol. The mixture is stored at 4°C for 12 hours to allow complete precipitate formation, then centrifuged. After drying to evaporate excess ethanol, the precipitated heparin is redissolved in aqueous solution, pH 7.0, filtered through a 0.2 micron Millipore filter, and relyophilized to dryness.

EXAMPLE IV

Effect of 2-0-Desulfated Heparin on Blood Coagulation:

The anti-coagulant potential of the desulfated heparin from Example III was studied by determining its effect on the activated partial thromboplastin time (APPT) in vitro. The test was performed in the usual fashion used to monitor the anti-coagulant effect of heparin clinically in patients. The test used 0.1 and 1.0 mg/ml heparin or heparin, 2-0-desulfated according to Example III, added to human test serum in vitro.

TABLE II

	Control	Heparin		2-0-Desulfated Heparin	
Conc. (mg/ml)	0	1.0	0.1	1.0	0.1
Time to Clot Formation (sec)	35-45	>150	80	42	38

The 2-0-desulfated heparin from Example III was

also studied to determine whether plasma dilutions of 0.1

mg/ml heparin or heparin, desulfated according to Example

III, inhibited factor Xa, prolonging test time in an assay

for Xa activity utilizing plasma treated with Russell viper

5   venom.

TABLE III

Dilution	Anti-factor Xa Activity		
	Control Plasma	Heparin	2-O-Desulfated Heparin
1:2		> 8 min.	42 sec.
1:10		> 7 min.	33 sec.
1:100		42 sec.	32 sec.
1:1000		32 sec.	32 sec.
0	35 sec.		

In contrast to heparin, the heparin desulfated

15   according to Example III showed little ability to prolong

the APTT and little antifactor Xa activity.

Thus, the 2-O-desulfated heparin showed a much reduced

anti-coagulant activity when compared to non-desulfated

heparin.

20   EXAMPLE V

Interaction of 2-O-Desulfated Heparin with HLE and

Cathepsin G:

The inhibition of HLE by 2-O-desulfated heparin

of Example III was measured by incubating a constant amount

25   of HLE (100 pmol) with increasing amounts of 2-O-desulfated

heparin (10-60 pmol, I/E ratio 0.1-0.6) for 30 minutes at

25°C in 500 microliters of Hepes buffer (0.125 M, 0.125%

Triton-X-100, pH 7.5) diluted to the final volume of 900

microliters. The remaining enzyme activity was measured by

30   adding 100 microliters of 3 mM N-Suc-Ala-Ala-Val-NA (Sigma

Chemicals, St. Louis, MO, made in dimethylsulfoxide [DMSO])

12

and reading the absorbance of the proteolytically released chromogen 4-Nitroaniline at 405 nm. The percentage of inhibition was calculated based on enzyme activity without inhibitor.

5   The inhibition of cathepsin G by 2-0-desulfated heparin of Example III was measured as described above, except that the substrate was 3 mM Suc-Ala-Ala-Pro-Phe-pNA (Sigma Chemical, in DMSO).

           Also, bovine ligament elastin was prepared

10   according to B. C. Starcher, Anal. Biochem. (1976) 74:441-447. The elastin was assessed for purity by amino acid analysis. Its degradation was assayed using elastin radiolabeled with ^{3}H-NaBH$_4$, following the methods described in P. J. Stone, et al., Methods Enzymol. (1982) 82:588-605.

15   The tritiated powdered elastin was homogenized and washed in phosphate buffered saline (PBS), pH 7.4 just before use to remove unincorporated radioactivity. A constant amount of human leukocyte elastase (HLE) (67 pmol) was pre-incubated with increasing amounts of 2-0-desulfated heparin

20   (6.7-134 pmol) at 37°C for 30 minutes in a final volume of 1.0 ml Hepes buffer (0.125 M, 0.125% Triton-X-100, pH 7.5). An aliquot of 900 microliters of each reaction mixture was incubated with 5 mg ^{3}H-elastin and 100 microliters of 0.9% saline at 37°C. Solubilized peptides

25   were separated from the elastin suspension by filtration through medium-porosity filter paper. The rate of degradation was determined by quantifying the solubilized ^{3}H peptides.

           The results of the interactions of 2-0-desulfated

heparin and heparin with HLE and cathepsin G are shown in Table IV.

TABLE IV

PERCENT INHIBITION

		HLE			Cathepsin G	
Substrate[a]		A		B		C
Inhibitor[b]	H	ODH	H	ODH	H	ODH
Ratio I:E						
0.1:1	23	20	72	73	31	14
0.2:1	81	74	95	77	49	54
0.3:1	88	84	-	-	-	-
0.4:1	87	85	92	72	67	56
0.5:1	87	84	94	85	-	-
0.6:1	99	100	-	-	-	-
0.8:1	-	-	-	-	78	69
1.0:1	-	-	97	85	76	69
2.0:1	-	-	97	84	76	67

[a] A = N-Suc-Ala-Ala-Val-NA; B = ^{3}H-Elastin; C = Suc-Ala-Ala-Pro-Phe-pNA.

[b] H = Heparin; ODH = 2-0-Desulfated Heparin according to Example III

As is shown in Table IV, ratios of inhibitor to substrate (I:E) of greater than 0.2 produces significant inhibition of human leukocyte elastase (HLE), I:E ratios of greater than 0.4 also provides significant inhibition of cathepsin G by both heparin and heparin 2-0-desulfated according to Example III. Throughout the range of ratios,

according to Example III. Throughout the range of ratios, there is very little difference between the effective inhibition of heparin and the heparin 2-0-desulfated according to Example III. Thus, the 2-0-desulfated heparin

5   provides substantially the same inhibition as does the non-desulfated heparin. In addition, the 2-0-desulfated heparin showed little anticoagulant activity. This is in contrast to the unmodified heparin which is a very active anticoagulation agent.

10   EXAMPLE VI

In Vivo Studies:

The ability of desulfated heparin to prevent human leukocyte elastase (HLE)-mediated lung injury was assessed in female golden Syrian hamsters (Harlan

15   Industries, Indianapolis, Indiana) weighing 90 to 110 g. Phenobarbital-anesthesitized hamsters were injected intratracheally with 0.25 ml sterile 0.9% saline (NS), 0.25 ml NS containing HLE (100 μg), or 0.25 ml NS containing 500 μg of heparin (Sigma) or 2-0-desulfated heparin according

20   to Example III followed by 0.25 ml NS with HLE. Animals were killed by exsanguination 24 hours after treatment. The throat was opened and lungs dissected en bloc. The trachea was cannulated with polyethylene tubing and lavaged with five sequential aliquots of 3 ml NS. Lavage fluid was

25   centrifuged at 200 Xg for 10 minutes. The resulting cell pellet was re-suspended in 1 ml Hank's balanced salt solution (HBSS) for performing cell count and differentials. The supernatant was assayed for protein and hemoglobin, as indices of acute injury. The results are

15

TABLE V

	PMN[a] (X10⁶ Cells)	Total Hemoglobin (mg)	Protein (mg/ml)
Injected Solution[b]			
Control 0.5 ml NS	0.95 (0.443)	0.396 (0.215)	35.08 (0.111)
HLE (100 μg)	16.3 (0.744)	8.15 (0.53)	100.69 (0.98)
HLE + H (100 μg + 500 μg)	10.83 (0.452)	0.867 (0.439)	41.06 (0.114)
HLE + ODH (100 μg + 500 μg)	9.83 (0.86)	1.5 (0.23)	65.80 (0.659)

---

[a] PMN = polymorphonuclear leukocyte

[b] HLE = human leukocyte elastase; H = heparin; ODH = 2-O-desulfated heparin according to Example III

Both heparin and 2-O-desulfated heparin, according to Example III, were potent inhibitors of elastase induced injury in vivo.

The 2-O-desulfated heparin from Example III has been tested for toxicity. Other sulfated polysaccharide inhibitors of elastase and cathepsin G, such as dextran sulfate, produced hemorrhage into lung air sacs (alveolar hemorrhage) when injected into rats intratracheally in doses as low as 0.5 mg/kg. The 2-O-desulfated heparin from Example III produced no alveolar hemorrhage in rats even in intratracheal doses of 10 mg/kg.

The alkaline hydrolyzed heparin produced according to Example III can be used to treat respiratory diseases such as emphysema, chronic bronchitis, Adult Respiratory Distress Syndrome (ARDS) and cystic fibrosis by administration to the respiratory tree directly as an

16

aerosol. A dose of from about 10 to about 100 mg of 2-0-desulfated heparin dissolved in 3 ml of sterile 0.9% saline is aerosolized into the lung about every 6 hours (or about 4 times daily) using any common clinically available aerosol device (such as a Devilbiss or Acorn Nebulizer) attached to a positive pressure source (either compressor or compressed air or oxygen) to generate aerosols of particles less than 10 microns mass median diameter.

The lower doses would be effective for diseases such as chronic bronchitis, whereas the higher doses would be needed for cystic fibrosis, in which the levels of elastase in respiratory secretions are much higher.

For treatment of diseases of ischemia-reperfusion such as myocardial infarction and stroke, the 2-0-desulfated heparin would be administered intravenously (IV) by continuous infusion. After a bolus loading dose of 0.5 mg/kg intravenously, 2.5-5.0 mg/kg of 2-0-desulfated heparin is mixed with 250-500 ml of 5% dextrose, 0.45% NaCl or 0.9% NaCl and infused continuously over 24 hours to maintain a constant blood level of drug within the vascular system.

The appended claims set forth various novel and useful features of the invention.

CLAIMS:

1. A medicament for the inhibition of neutrophil elastase and cathepsin G in mammals comprising a treatment effective amount of nonanticoagulant desulfated heparin prepared by removal of the 2-0-sulfate of α-L-idufonic acid-2-sulfate saccharide units of heparin by alkaline hydrolysis.

2. The medicament of Claim 1 wherein said medicament is administered by aerosolization.

3. The medicament of Claim 1 wherein said medicament is administered by intravenous (IV) injection.

4. The medicament of Claim 1 wherein the ratio of 2-0-desulfated heparin to elastase is greater than about 0.2 and less than about 2.0.

5. The medicament of Claim 1 wherein the ratio of 2-0-desulfated heparin to cathepsin G is greater than about 0.4 and less than about 2.0.

6. The medicament of Claim 1 including a physiologically acceptable carrier.

7. The medicament of Claim 6 wherein said carrier is selected from the group consisting of physiologically buffered saline, normal saline, and distilled water.

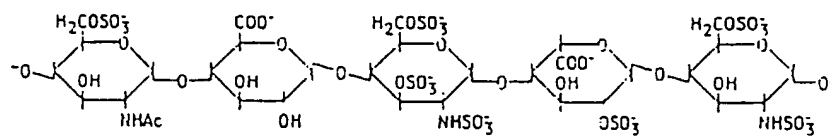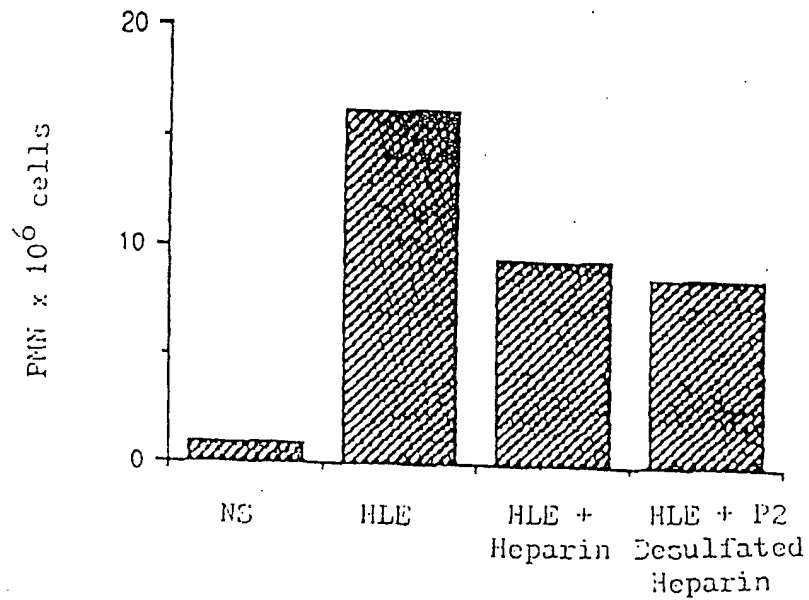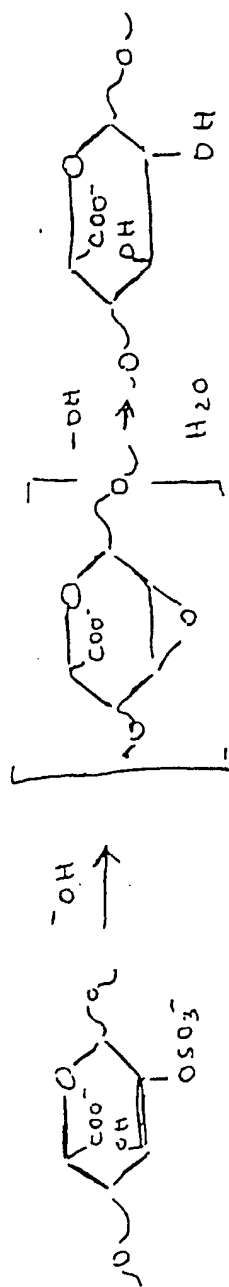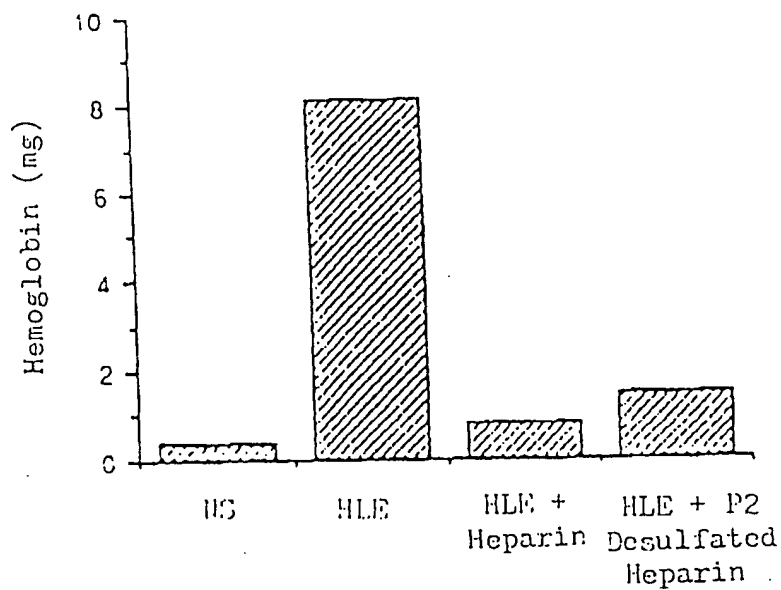8. A method for inhibiting neutrophil elastase and cathepsin G in mammals comprising administering a treatment effective amount of 2-0-desulfated heparin of Claim 1 to a mammal.

9. The method of Claim 8 wherein said treatment effective amount is administered by aerosolization.

18

10. The method of Claim 8 wherein said treatment effective amount is administered by intravenous (IV) injection.

11. The method of Claim 8 wherein the ratio of 2-O-desulfated heparin to elastase is greater than about 0.2 and less than about 2.0.

12. The method of Claim 8 wherein the ratio of 2-O-desulfated heparin to cathepsin G is greater than about 0.4 and less than about 2.0.

13. The method of Claim 8 including mixing said 2-O-desulfated heparin with a physiologically acceptable carrier.

14. The method of Claim 13 wherein said carrier is selected from the group consisting of physiologically buffered saline, normal saline, and distilled water.

Fig. 1

Fig. 3

Fig. 2

Fig. 4

Fig. 5